UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM:

To: Kable Davis, MS, PM03

From: Eric Bohnenblust, Ph.D., Entomologist

Secondary Review: Jennifer Saunders, Ph.D., Senior Biologist

Date: 3/20/2017

Subject: PRODUCT PERFORMANCE DATA EVALUATION RECORD (DER)

THIS DER DOES NOT CONTAIN CONFIDENTIAL BUSINESS INFORMATION

Note: MRIDs found to be **unacceptable** to support label claims should be removed from the data matrix.

DP barcode: 404456, 380444
Decision no.: Reregistration
Submission no: Reregistration
Action code: Reregistration
Product Name: Perimeter Insect Guard Insect Repellant Apparel
EPA Reg. No or File Symbol: 82392-1
Formulation Type: Impregnated Fabric
Ingredients statement from the label with PC codes included:
Permethrin 0.52% PC: 109701
Application rate(s) of product and each active ingredient (lbs. or gallons/1000 square feet or per acre as appropriate; and g/m^2 or mg/cm^2 or mg/kg body weight as appropriate): 0.52% permethrin equivalent to approximately 0.125 mg permethrin/cm^2 fabric, but varies based on weight of the fabric as seen in MRID 48883001 below.
Use Patterns: Treated fabrics for clothing, tents, truck covers, hunting blinds, and animal care products (e.g., pet blankets) to repel ticks, mosquitoes, spiders, ants, fleas, flies, chiggers, and midges.

I. Action Requested: Review 10 MRIDs submitted under reregistration to determine if they support efficacy of the product against ticks, mosquitoes, spiders, ants, fleas, flies, chiggers, and midges.

II. Background: The registrant submitted under Reregistration (PDCI-109701-26513) 10 MRIDs to support efficacy claims against ticks, mosquitoes, spiders, ants, fleas, flies, chiggers, and midges for EPA Reg. No. 82392-1. MRIDs 41536601, 41536602, and 41536603 were previously reviewed under DPs 424814 and 408028; detailed methods for these MRIDs can be found in the previous reviews and conclusions as they pertain to EPA Reg. No. 82392-1 are presented below. MRID 47634401 was previously reviewed in DP 360410; however, additional supplemental data were submitted (MRID 48047801) so this MRID is reviewed again to assess the effect of the new information. MRID 46533303 was previously reviewed in DP 318604, however, the previous review did not adequately describe the study methods and results, so this MRID is reviewed in more detail below. MRID 45551519 was previously reviewed under DP 424814; the previous review did not provide detailed information, thus this MRID is reviewed in detail below.

III. MRID Summary: (primary reviews are attached)

41536601. Interim Report on Contract for Further Investigation of the Application of Permethrin to Battle Dress Uniform (BDU).

(1) **Conclusion: Unacceptable.** This MRID describes the levels of permethrin impregnated on fabrics using different application methods. The application procedures described above are not the same as or similar to the application procedure for EPA Reg. No. 82392-1. There are no efficacy data presented to determine if the permethrin levels presented are of relevance from an efficacy standpoint.

41536602. Contract for Tests on Arthropod Repellent Impregnation.

(1) **Conclusion: Unacceptable.** This MRID describes the levels of permethrin impregnated on fabrics using different application methods. The laundry application method appears similar to the application procedure for EPA Reg. No. 82392-1, but is not the proprietary method provided by the registrant when the product was initially registered. The Agency requires product specific data to show permethrin retention and efficacy of the products associated with the proprietary impregnation method. There are no efficacy data presented to determine if the permethrin levels presented are of relevance from an efficacy standpoint.

41536603. Durability of Permethrin as a Potential Clothing Treatment to Protect Against Blood-Feeding Arthropods.

(1) **Conclusion: Supplemental.** This study does not, by itself, support efficacy of EPA Reg. No. 82392-1 against mosquitoes, ticks, or other arthropods. While the data presented here show that permethrin is capable of killing mosquitoes and flies through forced exposure at the rate labeled for EPA Reg. No. 82392-1, the data do not show whether the fabric will protect humans from being bitten by these arthropods. The study also shows that the higher treatment rates of 0.25 mg permethrin/cm^2 and above kill lone star ticks. Replication was not adequate in these studies. For treated fabric, the Agency requires efficacy studies with mosquitoes to be conducted with a human host with a measurement endpoint of bite protection. The Agency does not believe that forced exposure tests using mortality as a measurement endpoint and without a host are adequate to determine if an insect can bite a person or not. These products are intended to prevent insects from feeding on a host, not necessarily to kill the insect, and this type of study cannot support a bite protection endpoint. In addition, for any efficacy claims against ticks, data showing acceptable efficacy of the product against deer ticks, lone star ticks, and either American or brown dog ticks should be provided.

45551519. Permethrin-Treated Jackets Versus Repellent Treated Jackets and Hoods for Personal Protection Against Black Flies and Mosquitoes.

(1) non-GLP

(2) **Methods:** This MRID is a published study evaluating the efficacy of jackets and hoods treated with permethrin, DEET, or tetrahydrofuranfuryl octanoate (THFO) against black flies and mosquitoes. Fabric was either untreated, or treated with 0.25 g active ingredient (Deet or THFO)/g fabric, or 0.07 g permethrin/g fabric using an immersion application method. Efficacy was tested in Petawawa, ONT, Canada using six human subjects. None of the fabrics were subjected to laundering after treatment with repellent or permethrin. Subjects were exposed to natural populations of mosquitoes and black flies in the field. Species present at the testing location were: *Simulium venustum, Simulium decorum, Coquillettidia perturbans, Aedes vexans, Aedes cinereus, Aedes intrudens, Aedes sticticus,* and *Aedes stimulans* (note MRID was difficult to read so species names may be misspelled). Subjects were subjected to insects during the morning or evening, "for a period sufficient to accumulate a relatively large number of control and test landing counts with care taken to ensure that each test item and subject was exposed to the biting-fly population for the same length of time." Subjects tested each treatment in a randomized order with landing counts taken between each treatment period. This procedure was repeated at least 3 times with subjects wearing permethrin treated jackets located at least 50 m from subjects wearing untreated jackets. Efficacy was measured as the number of insect landings that occurred on the face, which was considered indicative of the relative effectiveness of individual garments. Data for landings which occurred on the hands or on the front of the jacket

were included for reference purposes. Percent efficacy was calculated using Abbotts formula.

(3) **Results:** The average number of insect landings per subject on the face was reduced by 76% with permethrin treated jackets and 91% with jackets treated with DEET when compared to the control treatment. The average number of insect landings on the front of the jackets was reduced by 70% for permethrin and 77% for DEET treatments compared to the control. For permethrin treated jackets, insect landings were only reduced by about approximately 40% (approximately 36 landings per subject) through the first ten minutes of the observation periods but during next five subsequent 10 minute intervals were between about 8-17 landings per subject. During the second through the 4th 10-minute intervals, insect landings on the untreated control were between 20-30 landings per subject, but in the 5^{th} interval jumped to over 60 landings per subject and in the 6^{th} interval were over 30 landings per subject. The authors suggest that the reduction in insect landings on the permethrin treated and untreated controls is indicative of a possible area-wide effect probably based on a knockdown/mortality effect.

(4) **Conclusion: Unacceptable.** This study does not support efficacy claims because efficacy of the permethrin treatment never reached 90%, the similarity between the tested fabric and EPA Reg. No. 82392-1 is unclear, fabrics were not impregnated using the same method as EPA Reg. No. 82392-1, and mosquito and black fly landings were combined so we cannot determine if the product was effective against one group or both groups. In addition, permethrin is not a volatile repellent, therefore measuring efficacy using landings on uncovered skin is not the best way to represent efficacy for permethrin treated fabrics. Sample size is also low and was not justified statistically.

46533303. Permethrin Retention and Efficacy on Sample Production for Proposed Product "Perimeter-2 Insect Guard."

(1) non-GLP.

(2) **Methods:** This MRID documents two studies, one testing efficacy of treated fabrics against mosquitoes, and the second one evaluating permethrin retention on treated fabrics.

Study 1, Permethrin Retention Study: The method of application (attachment 1) is product specific. This study used the AATC test method 143-2001: Appearance of Apparel and other Textile End Products after Repeated Home Laundering. The laundering method is based on a typical consumer laundering process. Permethrin retention on nine samples of unwashed treated fabric and 17 samples of washed treated fabric was assessed using GC-MS.

Study 2, Efficacy Study: This study evaluated military garments treated with permethrin by the registrant for efficacy against mosquitoes using a forced contact exposure assay. The contact exposure assay was conducted using 5 *Aedes aegypti* mosquitoes per replicate. Replication was not provided. Mosquitoes were exposed to treated fabric for two minutes and then assessed for knockdown at 15 and 60 minutes post exposure. Fabric was treated with 0.125 mg permethrin/cm^2 fabric and washed 25 times. A control treatment was not utilized.

(3) **Results:**

Study 1: The average amount of permethrin on unwashed treated fabric was 0.538% w/w, with a range of 0.25 – 0.81%. For the unwashed treated fabric samples, the percent of permethrin residues fall within the certified limits listed on the CSF for only two of the tested samples. The average amount of permethrin on treated fabric laundered 25 times was 0.412% w/w, with a range of 0.19 – 0.72%.

Study 2: Knockdown of *Ae. aegypti* mosquitoes was over 80% when the amount of permethrin on treated fabric was greater than 0.332% w/w with the exception of two individual samples. Although 14 of 36 samples contained less than 0.332% w/w of permethrin after 25 washes and % knockdown was less than 80% during tests with these samples, these data do not show a predictive relationship between percent knockdown and permethrin content. Moreover, the relationship between bite protection and percent knockdown is unclear and the Agency does not use knockdown forced exposure bioassays to support efficacy claims against mosquitoes for treated fabrics intended to be worn. Note on average, knockdown was greater than 80% on fabrics retaining at least 0.447% permethrin w/w. While this indicates the permethrin is bio-available and can have a knockdown effect using a forced exposure, this only pertains to original unwashed product for EPA Reg. No. 82392-1 despite fabric being laundered 25 times. In

over one third of the cases, laundering 25 times resulted in permethrin levels below 0.447% w/w, thus claims for 25 washes cannot be supported because the retention of permethrin is not consistent through 25 washes.

(4) **Conclusion: Unacceptable.** This study does not support efficacy claims for the proposed product because the fabric samples in the retention study do not conform to CSF specifications, Petri dish forced exposure assays are not acceptable, the data do not consistently show efficacy after 25 washings likely due in large part to the high variability in initial permethrin impregnation, and only one mosquito species was tested. For more information on the specific mosquito species required for any efficacy claims against mosquitoes please see https://www.epa.gov/pesticide-registration/guidance-efficacy-testing-pesticides-targeting-certain-invertebrate-pests.

48883001. Permethrin Retention and Efficacy on Sample Production for Product "Perimeter-2 Insect Guard."

(1) non-GLP

(2) **Methods:** This MRID documents three studies, one testing efficacy of treated fabrics against mosquitoes, one evaluating permethrin retention on treated fabrics, and one an independent evaluation of percent bite protection.

Study 1, Permethrin Retention Study: The method of application (attachment 1 in the MRID) is product specific. This study used the AATC test method 143-2001: Appearance of Apparel and other Textile End Products after Repeated Home Laundering. The laundering method is based on a typical consumer laundering process. Permethrin retention on nine samples of unwashed treated fabric and 17 samples of washed treated fabric was assessed using GC-MS. Note replication of retention analysis decreased with each washing interval.

Study 2, Efficacy Study: This study evaluated military garments treated with permethrin by the registrant for efficacy against mosquitoes using a forced contact exposure assay. The contact exposure assay was conducted with three replicates using 10-20 *Aedes aegypti* mosquitoes per replicate. Mosquitoes were exposed to treated fabric for two minutes and then assessed for knockdown at 15 and 60 minutes post exposure. Fabric was treated with 0.113-0.166 mg permethrin/cm^2 fabric and washed 0, 25 or 50 times. Note the mg permethrin/cm^2 fabric is different for fabrics consisting of different materials.

Study 3, Bite Protection: Methods were not provided for the percent bite protection study which evaluated efficacy against *Aedes aegypti* and *Anopheles albimanus*.

(3) **Results:**

Study 1: The average amount of permethrin on unwashed treated fabric was between 0.51-0.54% w/w depending on the fabric type. Of the unwashed treated fabric samples, the percent of permethrin residues did not fall within the certified limits for 5 samples, these samples were marginally over the certified limits and could be due to reasonable variations in the treatment methods. The average amount of permethrin on treated fabric laundered 25 times was 0.24 – 0.46% depending on the fabric type. The average amount of permethrin on treated fabric laundered 50 times was 0.14 – 0.36% depending on the fabric type. For more detailed results, see the tables in the attached primary review. Based on these data, noting the small sample size after 50 washes, the 55% cotton: 45% polyester twill garments provide the poorest retention of permethrin residues after 50 washes, followed by 50% cotton & 50% Nylon RipStop, Fire-Resistant Army Combat Uniforms (FRACU), and 50% cotton & 50% nylon twill.

Study 2: Control mortality was acceptable for both mosquito species for all washing regimens. At 15 minutes post exposure, knockdown of *Ae. aegypti* exposed to all unwashed treated fabrics except 55% cotton:45: polyester twill, was 79% or less. On the unwashed treated 55% cotton:45: polyester twill, knockdown of *Ae. aegypti* at 15 minutes post exposure was over 90%; this fabric had the highest initial levels of permethrin (averaging 0.141 – 0.166 mg permethrin/cm^2 fabric across the replicates). Knockdown of *Ae. aegypti* exposed to all treated fabrics washed 25 times was less than 50%, and for fabrics washed 50 times was less than 16%. Knockdown of *Ae. aegypti* for all fabric types and wash regimens was greater than 90% at one hour after exposure.

Study 3: Results were not evaluated because methods were not given and therefore this study cannot be used to

support any efficacy claims.

(4) **Conclusion: Supplemental.** The information regarding retention of permethrin residues on fabrics after washing suggests that different fabrics are impregnated with different amounts and retain residues differently. These data, although based on a small sample size, also suggest that the FRACU fabric is not a worst case scenario for permethrin retention. Although when physical characteristics are considered, the FRACU could possibly be the worst case scenario for bite protection with military uniforms. This study does not support efficacy claims because the forced exposure assay is not adequate to support efficacy claims against mosquitoes for treated clothing articles. The data also do not support efficacy claims for the non-wearable items (e.g., awnings, truck covers) which are more like residual spray uses and should be supported by data showing mortality. Also, the bite protection study provided is incomplete.

47634401. Perimeter Insect Guard Repellent Apparel: Product Performance.

(1) non-GLP

(2) **Methods:** This study documents an assay assessing bite protection of permethrin impregnated military uniforms against *Aedes aegypti* and *Anopheles albimanus* mosquitoes. This study was conducted with Human Subjects and therefore would require review by the Human studies review board. The reviewer did not do an in-depth review of the methods because more information is required regarding the actual conduct of the study. The protocol provided was a copy of the proposed protocol and not the protocol as conducted.

(3) **Results:** Summary data show greater than 95% bite protection against *Ae. aegypti* and *An. albimanus* for all garments through 50 washings.

(4) **Conclusion: Upgradable.** This study and supplement (MRID 48047801) as submitted do not support any efficacy claims for EPA Reg. No. 82392-1. This study was only conducted with four subjects. Adequate sample sizes for these types of studies are typically in the range of 8-10 subjects, but should be justified using a power analysis. Statistical justification for sample size selection is missing. Moreover, the amount of permethrin documented in the retention analyses should be provided on a w/w basis for the unlaundered treated fabrics for different uniform types to ensure that the product falls within specifications listed on the CSF. The Agency cannot rely on this study as presented because the study is missing details regarding methods used to conduct the study. The protocol submitted in the study was written as to how the study would be conducted in the future; however, a detailed protocol documenting how the study was actually conducted is necessary to review this study. The reviewer does not know if any deviations were made to the protocol during conduct of the study. In addition, no data are presented from the control tests to assess control bite through. For this study to be considered to support efficacy of the registered product, all methodological details must be submitted. Also, because the study only evaluated efficacy using four subjects, MRID 48256101 could not be used by itself, but would have to be accompanied by additional acceptable data showing efficacy of EPA Reg. No. 82392-1 against mosquitoes.

(5) **Special Note:** This study was conducted after the Human Studies Rule went into effect (April 7, 2006). Should the registrant decide to submit the information and methodology as outlined above, this study will need to be reviewed by the Human Studies Review Board.

48047801. Perimeter Insect Guard Repellent Apparel: Product Performance Supplemental Information to MRID No. 47634401.

(1) non-GLP

(2) **Conclusion: Upgradable.** This study provided supplemental information for MRID 47634401, for more detailed review of the information found in this supplement please see the review and conclusions for MRID 47634401 above.

45618839. Wear and Wash Persistence of Permethrin Used as a Clothing Treatment for Personal Protection Against the Lone Star Tick (Acari: Ixodidae).

(1) non-GLP

(2) **Methods:** This MRID is a published paper assessing the efficacy of permethrin treated clothing against lone star ticks and mosquitoes, and GC analysis of permethrin residues.

Lone Star Ticks: Unknown military uniforms were treated at a rate of 0.125 – 0.2 mg permethrin/cm^2 fabric using Permanone® 40% EC and Pramex 27.5% EC. Clothing was saturated using an unknown method and then held in a box for 24 hours to enhance penetration of the treatment in the clothing. After treatment uniforms were air dried. Untreated uniforms were used as an untreated control. One to three subjects wore the uniforms in tick infested environments and the number of dead adult ticks and nymphs were evaluated on treated clothing and the number of live ticks on untreated clothing. Uniforms were worn through 132 hours of wear or through 4 wash cycles.

Mosquitoes: Petri-dish style assays were used with either 2 minute or 30 second exposures of *Aedes aegypti* and *Anopheles quadrimaculatus*. Methods reference a previous paper and provide very few details. Replication was not provided.

Wear and Wash Stress: Clothing that was subjected to wear and wash tests in the studies with mosquitoes and lone star ticks above were analyzed for permethrin residues using GC-MS methods.

(3) **Results:**

Lone Star Ticks: Few dead adult lone star ticks were found on treated clothing regardless of wear time or number of washes. The mean number of dead nymphs on treated clothing was reduced by about 75% for unwashed treated clothing subjected to different wear durations when compared to untreated clothing. For the different washing regimens, the number of dead nymphs was reduced by over 90% for unwashed treated fabric and treated fabric washed 1 and 2 times in relation to the number of live tick nymphs found on untreated clothing. The number of dead ticks was reduced by less than 90% after 3 and 4 washes when compared to live ticks on the control subjects. The number of live ticks in the untreated control group was typically over 200 ticks/hour.

Mosquitoes: In the 2 minute constant exposure assay, the time to 100% knockdown for both mosquito species took 10 minutes or longer to occur for treated fabrics washed four times or worn for 132 hours.

Wear and Wash Stress: The amount of permethrin remaining after 132 hours of wear was 0.146-0.173 mg permethrin/cm^2 for permanone and was 0.175-0.207 mg permethrin/cm^2 for pramex. Treated fabric did not retain permethrin as well after washing as with wear. After 4 washes, 0.067-0.093 mg permethrin/cm^2 remained using the permanone treatment, and 0.069-0.127 mg permethrin/cm^2 remained using the pramex treatment.

(4) **Conclusion: Unacceptable.** This study only used one to three test subjects per treatment group, and the impregnation method was not the same as the method used for EPA Reg. No 82392-1, the mosquito bioassay is not adequate to support efficacy and efficacy against lone star ticks was not consistently over 90% for unwashed treated clothing.

41708401. Residual Effectiveness of an Insecticidal Spray Product on Three Types of Surface Material Against the Deer Tick, *Ixodes Dammini*.

(1) GLP status not provided.

(2) **Methods:** This laboratory study tested the efficacy against nymphs of deer ticks and brown dog ticks of a product containing 10% permethrin (EPA Reg. No. 4691-108) registered for surface application. An untreated control group was also tested. The product was applied to grass clippings, privet hedge, and short pile indoor/outdoor carpeting (some sort of artificial turf). Surfaces were replicated 5 times per tick species. The product was diluted to a 1% product per label instructions and then sprayed at a rate of 1 gal. dilution/750 ft^2 (5.0 ml of dilution/ft^2 or 3.63 g permethrin/750 ft^2). After treatment, parts of the surfaces were placed in 5 ml glass vials, and held for 7 days. Ten ticks of one species per vial were placed into each vial at 7 and 14 days post application and

assessed for mortality at 1, 2, 3, and 24 hours post exposure.

(3) **Results:** Mortality of both tick species was over 90% on all surfaces at 24 hours post initial exposure at both 7 and 14 days after application. Mortality in the control treatment was acceptable for all treatment groups except for the deer ticks exposed to privet at 7 days post application, where mortality was 18% in the control group.

(4) **Conclusion: Unacceptable.** This MRID does not support efficacy claims for EPA Reg. No. 82392-1 because the surfaces tested are not appropriate, the rate and application method are not the same as for EPA Reg. No. 82392-1, the tested product is not registered treating clothing or fabrics, exposure was forced for 24 hours, and tick nymphs were used instead of adults.

41712901. Residual Effectiveness of an Insecticidal Pet Spray Product on Three Types of Surface Material Against the Deer Tick, *Ixodes dammini*.

(1) **Conclusion: Unacceptable.** This MRID is identical to MRID 41708401, therefore, this MRID was not reviewed. For the conclusions pertaining to this MRID please see the conclusions for MRID 41708401.

IV. EXECUTIVE DATA SUMMARY:

(A) The submitted data does not support any pests or uses for EPA Reg. No. 82392-1.

V. LABEL RECOMMENDATIONS:

(1) All uses and pests should be removed from the label because they are not supported by data.

(2) The following marketing claims are acceptable: None.

(3) The following marketing claims are unacceptable: All.

(4) The following MRIDs should be removed from the data matrix, as they are classified as “unacceptable or supplemental” to support the product: 41536601, 41536602, 41536603, 45551519, 46533303, 47634401, 48883001, 41708401, 41712901, 48047801

(5) Note to PM/RM: The samples tested for permethrin retention in MRID 46533303 did not fall within the certified limits listed on the CSF for EPA Reg. No. 82392-1. The samples were impregnated using the product specific proprietary method provided for permethrin impregnation at the time of initial registration.

TASK 2 DATA EVALUATION RECORD

STUDY TYPE: Product Performance

MRID 488830-01. Lack, R. Permethrin Retention and Efficacy on Sample Production for Product "Perimeter-2 Insect Guard." May 1, 2012.

OCSPP Product Performance Guideline: 810.3700

Product Name: Perimeter-2 Insect Guard
EPA Reg. No. or File Symbol: N/A
Decision number: N/A
DP number: N/A

Prepared for
Registration Division (7505)
Office of Pesticide Programs
U.S. Environmental Protection Agency
Washington, DC 20460

Prepared by
Summitec Corporation
Task Order No.: 2-282

Primary Reviewer:
Chris Peterson, Ph.D.

Signature: Chris Peterson AE
Date: 12/21/2015

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Quality Assurance:
Angela M. Edmonds, B.S.

Signature: Angela M. Edmonds
Date: 12/21/2015

Disclaimer

This review may have been altered subsequent to the contractors' signatures above.
Summitec Corp. for the U.S. Environmental Protection Agency under Contract No. EP-W-11-014

EFFICACY STUDY DATA EVALUATION RECORD (REREGISTRATION)

Primary Reviewer's Name/Title: Chris Peterson, Toxicologist

STUDY TYPE:		PRODUCT PERFORMANCE: OCSPP 810.3700
MRID:		488830-01. Lack, R. Permethrin Retention and Efficacy on Sample Production for Product "Perimeter-2 Insect Guard." May 1, 2012.
TYPE OF DATA CALL IN:		PDCI
TESTING FACILITY:		Benzon Research, 7 Kuhn Dr., Carlisle, PA 17015; PineBelt Processing Company, 113 Fellowship Road, P.O. Box 557, Taylorsville, MS 39168
STUDY DIRECTOR or INVESTIGATOR:		Ron Lack, Study Director
SUBMITTER:		Ron Lack, Study Director
STUDY COMPLETED:		01/07/2012
CONFIDENTIALITY CLAIMS:		None
GOOD LABORATORY PRACTICE STUDY?:		This study was carried out to meet the Good Laboratory Practice Standard in 40 CRF Part 160 except for the following: PineBelt Processing, Inc. does not have a quality assurance team that monitored Benzon Research or its own facitlity.
TEST DETAILS:		• ACTIVE INGREDIENT NAME: See test materials section • A.I. %: See test materials section • PC CODE: 109701 • CAS NO: 52645-53-1 • FORMULATION TYPE: Liquid
APPLICATION RATES:		• TEST PRODUCT APPLICATION RATE(S): See test materials section • TEST PRODUCT ACTIVE INGREDIENT APPLICATION RATE(S): See test methods section
PEST(s) TESTED:		Yellow fever mosquito, *Aedes aegypti*

Efficacy Study Data Evaluation Record - Reregistration

Title: Permethrin Retention and Efficacy on Sample Production for Product "Perimeter-2 Insect Guard."

Purpose/Objective:

STUDY DESIGN: This study was carried out to determine the permethrin retention in military garments after repeated washings and the residual permethrin's ability to cause knockdown of mosquitoes after a two-minute exposure time.

Materials and Methods

Test Material(s):

SYNONYM: Permethrin, mixed cis, trans, Permanone 40 EC (40% permethrin & 60% other), CAS No. 52645-53-1

Permanone 40 EC was applied to fabric at 0.52% Permethrin by weight of fabric for four different fabric types. Permethrin concentration (average of all fabrics) was 0.130 mg/square cm Permethrin initially, 0.074 mg/square cm Permethrin after 25 washings (when the first bioassays were conducted) and 0.055 mg/square cm Permethrin after 50 washings (when the second bioassays were conducted).

Test Location: Carlisle, PA

Positive Control/Reference Standard, if used: Not used

Species Tested:

- Common name and scientific name of each species. Yellow fever mosquito, *Aedes aegypti*
- Life stage as egg or nymph or larvae including stadia; or adult and sex and age. Non-blood fed females, 5 to 15 days post emergence
- Describe the insecticide susceptibility status of the test population. Not reported
- Describe the origin of field collected strains. Not reported
- If female adults are used - are they gravid? Not reported
- Describe rearing techniques. Not described

Experiment description:

- List the treatments including the untreated control:

SYNONYM: Permethrin, mixed cis, trans, Permanone 40 EC (40% permethrin & 60% other), CAS No. 52645-53-1

Permanone 40 EC was applied to fabric at 0.52% Permethrin by weight of fabric for four different fabric types. Permethrin concentration (average of all fabrics) was 0.130 mg/square cm Permethrin initially, 0.074 mg/square cm Permethrin after 25 washings (when the first bioassays were conducted) and 0.055 mg/square cm Permethrin after 50 washings (when the second bioassays were conducted).

Mosquitoes were exposed to 6 by 6 inch squares of fabric.

Untreated control replicates consisted of mosquitoes exposed to untreated fabric.

An independent confirmation study was conducted at the Natick Soldier Research, Development and Engineering Center, Natick, MA

- Include a description of:
  - Test arenas and/or apparatus (include site description and location):
    starved prior to testing. Fabric samples tested were 6-inch (15 cm) squares laid in the inverted lid of petri plates. Ten to twenty mosquitoes were discharged into the petri plates through a hole in the bottom plate. The hole was sealed with tape and the mosquitoes were exposed to the fabric for 2 minutes at room temperature after which the fabric was removed and the mosquitoes remained in the petri plates for examination. Knockdown is defined as
  - Method(s) of application: Surface treatment
  - Number of replicates per treatment: 3 garments, two washed 0 and 25 times, one washed 50 times
  - Number of individuals per replicate: 10 to 20
  - Length of exposure to treatment (time in seconds, minutes or hours): 2 min
  - Were tested specimens transferred to clean containers?: No, but treated fabric was removed after 2 min exposure
  - Experimental conditions (state relative humidity, temperature, and photoperiod): Room temperature (data not presented)
  - The type of harborage if used in the experiment: Mosquitoes remained in the Petri dishes
  - The data and/or endpoints that were recorded and how they were assessed (e.g., prodded with a needle to see if specimens move): Knockdown 15 and 60 min after exposure to four different fabric types washed 0, 25 or 50 times
    and the mosquitoes remained in the petri plates for examination. Knockdown is defined as the inability to walk or fly in a coordinated manner. Complete laboratory reports are found

    The independent data reported "bite protection" against *Aedes aegypti* and *Anopheles albimanus*, but the testing methods and endpoints were not defined.

  - Report if morbidity and mortality were recorded separately: Not recorded separately
  - Statistical analysis conducted and justification for selecting the approach to data analysis and statistics used (were data corrected to account for abnormalities in the

data/study design, what level of significance was used, what were the confidence intervals around the mean value(s), was a median value also reported?): Not performed

Data Reported/Results

TABLE 2. TREATED FABRIC BIOASSAY RESULTS FOR 25 WASHES

	Rep 1(9/21/2011) % knockdown		Rep 1(9/21/2011) % knockdown		Rep 1(9/21/2011) % knockdown		Mean % knockdown	
	15 m	60 m	15 m	60 m	15 m	60 m	15 m	60 m
PROJECT 1								
1000 Control	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	**0.0**
1001-A (0-washes)	41.2	100.0	66.7	100.0	40.0	100.0	**49.3**	**100.0**
1001-B (25-washes)	36.8	100.0	55.0	100.0	40.0	100.0	**43.9**	**100.0**
1002-A (0-washes)	80.0	100.0	85.0	100.0	61.1	100.0	**75.4**	**100.0**
1002-B (25-washes)	50.0	100.0	65.0	95.0	52.6	100.0	**55.9**	**98.3**
PROJECT 2								
2000 Control	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	**0.0**
2001-A (0-washes)	56.3	93.8	70.6	100.0	63.2	100.0	**63.3**	**97.9**
2001-B (25-washes)	33.3	93.3	26.7	100.0	42.3	100.0	**34.1**	**97.8**
2002-A (0-washes)	33.3	100.0	52.9	100.0	56.3	93.8	**47.5**	**97.9**
2002-B (25-washes)	42.9	100.0	36.4	100.0	17.4	69.6	**32.2**	**89.9**
PROJECT3								
3000 Control	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	**0.0**
3001-A (0-washes)	70.0	100.0	94.1	100.0	58.3	100.0	**74.2**	**100.0**
3001-B (25-washes)	26.1	100.0	35.3	100.0	41.2	100.0	**34.2**	**100.0**
3002-A (0-washes)	76.5	100.0	64.7	100.0	41.2	100.0	**60.8**	**100.0**
3002-B (25-washes)	27.8	94.4	39.1	91.3	52.9	88.2	**39.9**	**91.3**
PROJECT 4								
4000 Control	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	**0.0**
4001-A (0-washes)	95.5	100.0	77.3	100.0	100.0	100.0	**90.9**	**100.0**
4001-B (25-washes)	38.9	100.0	25.0	100.0	33.3	94.4	**32.4**	**98.1**
4002-A (0-washes)	94.7	100.0	84.2	100.0	91.3	100.0	**90.1**	**100.0**
4002-B (25-washes)	44.4	100.0	56.0	100.0	25.0	100.0	**41.8**	**100.0**

TABLE 3 TREATED FABRIC BIOASSAY RESULTS FOR 50 WASHES

	Rep 1(9/21/2011) % knockdown		Rep 1(9/21/2011) % knockdown		Rep 1(9/21/2011) % knockdown		Mean % knockdown	
	15 m	60 m	15 m	60 m	15 m	60 m	15 m	60 m
PROJECT 1								
1000 Control	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	**0.0**
1003-A (0-washes)	83.3	100.0	62.5	100.0	72.2	100.0	**72.7**	**100.0**
1003-B (50-washes)	6.7	100.0	6.3	93.8	10.5	94.7	**7.8**	**96.2**
PROJECT 2								
2000 Control	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	**0.0**
2003-A (0-washes)	80.0	100.0	47.8	100.0	70.6	100.0	**66.1**	**100.0**
2003-B (50-washes)	25.0	100.0	11.1	94.4	5.9	88.2	**14.0**	**94.2**
PROJECT3								
3000 Control	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	**0.0**
3003-A (0-washes)	87.5	100.0	75.0	100.0	75.0	100.0	**79.2**	**100.0**
3003-B (50-washes)	16.7	100.0	11.8	70.6	19.0	95.2	**15.8**	**88.6**
PROJECT 4								
4000 Control	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	**0.0**
4003-A (0-washes)	100.0	100.0	94.4	100.0	100.0	100.0	**98.1**	**100.0**
4003-B (50-washes)	5.9	94.1	0.0	94.1	4.0	92.0	**3.3**	**93.4**

Fabrics for Projects 1 through 4 were, respectively:

a) Fire Resistant Army Combat Uniform (FRACU) (65% Rayon, 25% Para-Aramid, 10% Nylon)
b) Twill constructed fabric (50% cotton/50% nylon)
c) RipStop constructed fabric (50% cotton/50% nylon)
d) Twill constructed fabric (55% cotton/45% polyester)

Independent confirmation data submitted from Natick Soldier Research, Development and Engineering Center, Natick, MA:

Sample Set	#	Initial			20 Launderings			50 Launderings		
		mg/cm²	%Bite Protection		mg/cm²	%Bite Protection		mg/cm²	%Bite Protection	
			Ae. Aeg.	An. Alb.		Ae. Aeg.	An. Alb.		Ae. Aeg.	An. Alb.
Army-W-Lot#1-Oct10-ACUP-FAT-Coat-1	1	0.126	100.0%	100.0%	0.027	100.0%	100.0%	0.019	100.0%	100.0%
	2	0.127	100.0%	100.0%	0.028	100.0%	100.0%	0.180	97.0%	90.5%
	3	0.134	100.0%	100.0%	0.032	100.0%	100.0%	0.018	100.0%	95.6%
	AVG.	**0.129**	**100.0%**	**100.0%**	**0.029**	**100.0%**	**100.0%**	**0.072**	**99.0%**	**95.4%**
Army-W-Lot#1-Oct10-ACUP-FAT-Coat-2	1	0.120	100.0%	100.0%	0.029	100.0%	100.0%	0.019	100.0%	100.0%
	2	0.131	100.0%	100.0%	0.032	100.0%	100.0%	0.018	100.0%	100.0%
	3	0.139	100.0%	100.0%	0.030	100.0%	100.0%	0.018	100.0%	100.0%
	AVG.	**0.130**	**100.0%**	**100.0%**	**0.030**	**100.0%**	**100.0%**	**0.018**	**100.0%**	**100.0%**
Army-W-Lot#1-Oct10-ACUP-FAT-Coat-3	1	0.135	100.0%	100.0%	0.032	100.0%	100.0%	0.019	100.0%	100.0%
	2	0.111	100.0%	100.0%	0.030	100.0%	100.0%	0.020	100.0%	100.0%
	3	0.141	98.3%	100.0%	0.033	100.0%	100.0%	0.020	100.0%	100.0%
	AVG.	**0.129**	**99.4%**	**100.0%**	**0.032**	**100.0%**	**100.0%**	**0.020**	**100.0%**	**100.0%**
Army-W-Lot#1-Oct10-ACUP-FAT-Trouser-3	1	0.105	100.0%	100.0%	0.006	100.0%	100.0%	0.013	100.0%	100.0%
	2	0.104	100.0%	100.0%	0.019	100.0%	100.0%	0.013	100.0%	100.0%
	3	0.120	100.0%	100.0%	0.019	98.1%	100.0%	0.014	100.0%	100.0%
	AVG.	**0.110**	**100.0%**	**100.0%**	**0.015**	**99.4%**	**100.0%**	**0.013**	**100.0%**	**100.0%**
Army-W-Lot#1-Oct10-ACUP-FAT-Trouser-4	1	0.104	100.0%	100.0%	0.027	100.0%	100.0%	0.021	100.0%	100.0%
	2	0.091	100.0%	100.0%	0.027	100.0%	100.0%	0.022	100.0%	100.0%
	3	0.109	100.0%	100.0%	0.027	100.0%	100.0%	0.023	100.0%	100.0%
	AVG.	**0.101**	**100.0%**	**100.0%**	**0.027**	**100.0%**	**100.0%**	**0.022**	**100.0%**	**100.0%**
Army-W-Lot#1-Oct10-ACUP-FAT-Trouser-5	1	0.131	100.0%	100.0%	0.028	100.0%	100.0%	0.023	100.0%	100.0%
	2	0.111	100.0%	100.0%	0.028	100.0%	100.0%	0.022	100.0%	100.0%
	3	0.102	100.0%	100.0%	0.027	100.0%	100.0%	0.022	100.0%	100.0%
	AVG.	**0.115**	**100.0%**	**100.0%**	**0.028**	**100.0%**	**100.0%**	**0.022**	**100.0%**	**100.0%**
		Avg.	**99.8%**	**100.0%**		**100.0%**	**100.0%**		**99.7%**	**98.5%**
	Total %Bite Avg.		**99.9%**			**100.0%**			**99.1%**	

- Deviations or amendments from the protocol. None reported
- For each tested species, report the % efficacy (e.g. knockdown, mortality, repellency) for each treatment group. Include the following information, if applicable:
    - Timepoints (e.g., 4 h, 24 h) at which greater than 90% efficacy was observed. 0 washings: 15 min for fabric 4, 60 min for all other fabrics; 25 washings: 60 minutes for all fabrics; 50 washings: 60 min for all fabrics except fabric 3. Independent data: Time to “bite protection” not specified
    - Tested a.i. application rate: 0.130 mg/square cm Permethrin initially, 0.074 mg/square cm Permethrin after 25 washings and 0.055 mg/square cm Permethrin after 50 washings
    - Surface tested, for residual studies (e.g. ceramic tile, wood panel): Fabric, washed 0, 25 or 50 times
    - Formulation type (e.g. aerosol, granular): Liquid
    - Application type (e.g. direct, surface, area): Surface
    - Timepoints at which corresponding control mortality is greater than 10%. Not observed

- Treatment of all four fabric types at 0.52% Permethrin by weight, a.i. rate of 0.130 mg/square cm Permethrin caused ≥90% knockdown within 15 min on fabric 4 and within 60 min on all other fabrics.
- After 25 washings, a residual of 0.074 mg/square cm Permethrin caused ≥90% knockdown within 60 minutes on all fabrics.
- After 50 washings, a residual of 0.055 mg/square cm Permethrin caused ≥90% knockdown within 60 minutes for all fabrics except fabric 3.
- In an independent confirmation study, treated fabrics provided ≥90% bite protection after 0, 20 and 50 washings.

- The methods for the independent confirmation study were not provided.
- Many of the Attachments are incorrectly cross referenced in the text. For example, on pg. 7 the reader is referred to Attachment D for the knockdown bioassay methods, but Attachment D is actually the Permethrin extraction content report. The attachments are properly listed on pg. 13.